Various modes of nucleic acid processing by mesophilic bacterial Argonaute proteins

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Argonaute (Ago) proteins are key components of RNA interference in eukaryotes, where they function as site-specific RNA nucleases guided by small noncoding RNAs and participate in post-transcriptional regulation. Many bacteria also encode Ago proteins but their functions and the mechanisms of action in bacterial cells remain unclear. We characterize *in vitro* biochemical properties of Ago proteins from several mesophilic bacteria, which can potentially be used as a tool for genome editing. Some of these proteins can bind small guide DNAs and act as DNA-dependent DNA nucleases, preferably acting on single-stranded DNA targets. We show that the preferred length of the guide DNAs bound by the Ago nucleases is 15-20 nucleotides, and that single-nucleotide mismatches between the guide and target DNA strands can significantly affect the slicing activity, depending on the mismatch position. In particular, mismatches in the 3'-supplementary guide region decrease the cleavage efficiency, while mismatches in the seed region have a mild effect on the target cleavage. 5'-Phosphorylation of guides increases the rate and accuracy of target cleavage. The Ago proteins can catalyze DNA cleavage at the physiological range of temperatures (from < 25 ºC to 60 ºC), depending on the type and concentration of divalent cations in the reaction. Interestingly, some Agos can also utilize small RNA guides and/or cleave RNA targets. Thus, mesophilic Ago nucleases that have slicer activity at physiological temperatures are perspective candidates for development of new tools for manipulation with nucleic acids. This work was supported by the grant 14.W03.31.0007 of the Ministry of Science and Higher Education of the Russian Federation.